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The Regulation of Embryo Implantation and Endometrial Decidualization by Progesterone Receptor Signaling

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Abstract

During the early stages of pregnancy, fertilized embryos must attach to the uterine epithelium, invade into the underlying uterine stroma, and the stroma must then differentiate in a process termed decidualization in order for a successful pregnancy to be initiated. The steroid hormone progesterone (P4) is an integral mediator of these early pregnancy events, exerting its effects via the progesterone receptor (PR). Insights gained from the use of mouse models and genomic profiling has identified many of the key molecules enlisted by PR to execute the paradigm of early pregnancy. This review describes several of the molecules through which the PR exerts its pleiotropic effects including ligands, receptors, chaperones, signaling proteins and transcription factors. Understanding these molecules and their concatenation is of vital importance to our ability to clinically treat reproductive health problems like infertility and endometriosis.

Keywords

Progesterone; Progesterone Receptor; Attachment; Implantation; Decidualization; Mouse Models

1. Introduction

According to the most recent data from the Centers for Disease Control and Prevention's National Survey of Family Growth, infertility is estimated to affect 2.1 million married women in the US. When the parameters describing unsuccessful pregnancy events are expanded to include reduced fecundity, defined as difficulties becoming pregnant or carrying a baby to term, it was estimated to affect 7.4 million married women, demonstrating the tremendous impact fertility problems have on female reproductive health. Although society and families have benefited from advancements in the field of assisted reproductive technology (ART), to which approximately 1% of US births can now be attributed to, the overall success rate of ART still remains low [Ola and Li 2006]. Many of the unsuccessful ART pregnancies can likely be attributed to the transfer of embryos into a nonreceptive uterus. In fact, recurrent implantation failure is considered to be an important

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limiting factor in the establishment of pregnancy by either ART or natural means [Norwitz et al., 2001].

In order for a successful pregnancy to be initiated, the maturation of a fertilized egg to an implantation-competent blastocyst must occur in precise synchrony with the succession of events that occur in the maternal uterus that render it receptive. This precise timing event has been termed 'the window of receptivity' and has been shown to be under the control of the steroid hormone estrogen (E2) [Ma et al., 2003]. During the window of receptivity, the trophectoderm layer of the blastocyst becomes juxtaposed with the uterine luminal epithelium in a process termed embryo attachment. Following attachment, the blastocyst invades through the luminal epithelium and into the underlying stroma and this process is called implantation. In most mammals, implantation triggers a rapid remodeling of the uterine stromal compartment resulting in a morphological and functional transformation including stromal cell proliferation and subsequent differentiation into large epithelioid cells in a process known as decidualization (reviewed in [Lee and DeMayo 2004]).

In humans and other menstruating primates, decidualization occurs independent of the presence of a conceptus during the midsecretory phase of the cycle following the postovulatory rise in progesterone [Finn 1987; Finn 1998]. The hallmarks of this differentiation and remodeling of the uterine endometrium in preparation of pregnancy are characterized by the transformation of the stromal cell to a rounded epithelioid cell secreting products such as prolactin (PRL) and insulin-like growth factor binding protein-1 (IGFBP-1), among many other factors [Daly et al., 1983; Gellersen et al., 2007; Irwin et al., 1989]. Decidualization is a progressive event, initiating around the spiral arteries of the superficial endometrium and eventually extending throughout the endometrium to the basal layer (reviewed in [Brosens et al., 2002]). The decidua plays a critical role in regulating trophoblast invasion, modulating the local immune response at the feto-maternal interface, and development of the placenta. Thus, an aberrant response of the endometrium to ovarian hormones results in a block in the initiation and progression of pregnancy and defective formation of the placenta, both of which are associated with recurrent miscarriages and infertility. Although E2 regulates the window of receptivity, progesterone (P4), produced by the corpus luteum following ovulation, is the predominant mediator of these events necessary for pregnancy (reviewed in [Franco et al., 2008; Lee and DeMayo 2004]).

The word progesterone was coined in the 1930s and is derived from the Latin *pro* and *gestare* to describe a substance which favors gestation. Indeed, P4 not only favors gestation, but it is absolutely required for it. Studies ablating P4 signaling either genetically or pharmaceutically demonstrate the paramount role of this steroid hormone and its signaling paradigm in pregnancy [Baulieu 1989; Baulieu 1991; Cheon et al., 2002; Lydon et al., 1995]. RU486, or mifepristone, was discovered in the early 1980's to be the first known P4 antagonist. Studies later determined that the administration of RU486 inhibited P4-mediated gene transcription, which, if administered postimplantation, ultimately lead to conceptus abortion [Baulieu 1989]. The abortifacient properties of RU486 later lead to its use as an emergency contraceptive, demonstrating the critical role P4 signaling plays in pregnancy [WHO 1999].

P4 exerts its effects by binding its cognate receptor, the progesterone receptor (PR), which is a member of the nuclear receptor superfamily of transcription factors. There are two predominant isoforms of PR, PR-A and PR-B, each of which is transcribed from alternative start sites within the same gene (reviewed in [Li and O'Malley 2003]). Although PR-B is the larger of the two isoforms, containing an additional 164 N-terminal amino acids, PR-A is the predominant functional isoform in the uterus [Conneely and Lydon 2000; Conneely et al., 2001; Conneely et al., 2003; Mulac-Jericevic et al., 2000; Mulac-Jericevic et al., 2003]. The

PR knockout (PRKO) mouse, lacking both PR-A and PR-B, displayed pleiotropic reproductive abnormalities and was shown to be infertile due to defects in multiple reproductive tissues including embryo implantation, uterine decidualization, and an abnormal response to E2 and P4 treatment [Lydon et al., 1995]. When individual PR-A and PR-B null mice (PRAKO and PRBKO, respectively) were generated, only the PRAKO female mice recapitulated the phenotype observed in PRKO mice; while in contrast, the PRBKO female mice were fertile [Conneely and Lydon 2000; Conneely et al., 2001; Conneely et al., 2003; Mulac-Jericevic et al., 2000; Mulac-Jericevic et al., 2003].

In humans, as in mice, both isoforms of PR are induced by estrogen and can be found in the epithelial and stromal compartments during the proliferative stage of the cycle [Koshiyama et al., 1995; Wang et al., 1994]. Transitioning into the secretory phase and during decidualization, PR-B is down-regulated in both compartments, while PR-A expression persists in the stromal compartment, indicating PR-A is the predominant mediator of decidualization in humans [Brosens et al., 1999; Wang et al., 1998]. Furthermore, it has been shown *in vitro* that PR-A is a stronger activator of decidual marker IGFBP-1 than PR-B [Gao et al., 2000]. However, the potential importance of PR-B should not be overlooked, because the ratio of PR-A to PR-B has been shown to play an important role in endometrial dysfunctions such as recurrent early pregnancy loss, endometriosis and endometrial cancer [Attia et al., 2000; Balleine et al., 2004; Carranza-Lira et al., 2000].

With advancements in gene targeting techniques and tools for genomic comparisons, many of the P4 mediators and regulators of pregnancy have been identified. With an emphasis on insights gained from mouse models, we herein concisely and comprehensively collate the prominent work thus far regarding P4 signaling, its modulators and effectors, and how this signaling axis regulates embryo implantation and subsequent decidualization (Table 1).

2. Mediators of PR Action

Execution of the P4/PR signaling paradigm of gestational regulation occurs via the manipulation of multiple levels of cellular and molecular biology. Molecules that affect the expression of the PR itself, those that regulate the PR's aptitude to respond to signals and the coregulators that enhance the PR's ability to activate transcription of its target genes all play important roles in the immediate initiation of the P4 response. In the following section, we focus on those molecules that facilitate PR signaling.

2.1 Steroid Hormone Regulation of PR Expression

One would be remiss to discuss the molecular mediators of pregnancy without first emphasizing the paramount role of the steroid hormones E2 and P4. The expression of both estrogen receptor (ER) and PR can be found in all compartments of the uterus, and embryo implantation has been shown to be critically dependent on their intricate balance and interaction [Rubel et al., 2010; Tan et al., 1999; Tibbetts et al., 1998]. On days 1 and 2 of pregnancy, preovulatory E2 stimulates uterine epithelial cell proliferation and growth. Then beginning on day 2.5 of pregnancy, luteal P4 attenuates this epithelial proliferation, promoting receptivity, and drives stromal proliferation. This PR-mediated transition from epithelial to stromal cell proliferation is requisite for successful implantation as it's been shown in several mouse models that a failure to do so results in a block in embryo attachment. While the role of PR was previously discussed herein, it should be noted that in the presence of P4, a negative-feedback loop manifests to down-regulate the expression of the PR, occurring most notably in the glandular epithelium. Thus, PR can regulate its own transcription [Tibbetts et al., 1998].

On the other hand, follicular E2 causes a transition in which the expression of epithelial PR is repressed while stromal PR expression is induced [Curtis Hewitt et al., 2002; Kastner et al., 1990; Kurita et al., 2000; Tibbetts et al., 1998]. Previous experiments utilizing neonatal tissue recombination models suggested that this E2 regulation of PR expression was dependent on stromal ER α [Kurita et al., 2000]. These findings were recently confirmed when the E2-dependent redistribution of PR expression remained intact in the *Wnt7a*-Cre *ER α ^{fl/fl}* mouse model containing epithelial-specific ablation of *ER α* [Winuthayanon et al., 2010]. Like PR, ER consists of two predominant isoforms, *ER α* (*Esr1*) and *ER β* (*Esr2*). *ER α* appears to be the dominant mediator of uterine reproductive functions as knockout female mice for this isoform (*ER α KO*) were infertile while *ER β* knockout (*ER β KO*) females did not exhibit impairments in receptivity [Curtis et al., 1999; Kregge et al., 1998]. Interestingly, although *ER β KO* females were shown to be indistinguishable from wild type uteri on a molecular level, other studies indicate that ER β regulates ER α expression and its loss can render phenotypic differences, causing the role of this ER isoform to remain unclear [Hewitt et al., 2003; Wada-Hiraike et al., 2006; Weihua et al., 2000]. Despite a potential modulatory role of ER β , the complete infertility observed in *ER α KO* mice demonstrates that *ER α* is the prevailing mediator of E2 signaling in the uterus.

2.2 Regulation of PR Aptitude

P4 and PR regulate a plethora of different gene targets [Jeong et al., 2005]. Much of the specificity of PR target induction is dictated by the cellular context and interactions with other proteins. In the absence of P4, PR is found in the cytoplasm bound by a complex of heat shock proteins and immunophilins [Smith 2000]. The formation of such complexes functions to maintain receptor competence for hormone binding and transcriptional activation. *Fkbp4* (*Fkbp52*) and *Fkbp5* (*Fkbp51*), the expression of which overlaps with PR in the uterine stroma during implantation, are two immunophilins that have been shown to interact with PR. Although *Fkbp5* knockout mice do not present with any overt reproductive phenotype, both male and female *Fkbp4* knockout mice are infertile [Tranguch et al., 2005; Yang et al., 2006]. The fertility defect observed in these females was attributed to a complete failure in implantation and decidualization. Because *Fkbp4* acts to maintain PR competency, the molecular basis of this infertility was shown to be due to dramatically reduced PR transcriptional activity, and thus reduced expression of many known target genes and mediators of fertility including Homeobox A-10 (*Hoxa10*), Indian Hedgehog (*Ihh*), and Amphiregulin (*Areg*). Additionally, it's well known that one of the functions of PR is to suppress epithelial E2 sensitivity and proliferation, a process which is necessary to permit embryo attachment to the luminal epithelium [Conneely et al., 2001; Lee et al., 2010]. *Fkbp4* null females exhibit a failure of this process as indicated by elevation of E2 target genes such as lactoferrin (*Ltf*). Interestingly, P4 supplementation, although in a background dependant manner, was able to restore many of the defects observed in these mice. These results expose this mouse model as a good surrogate for studying P4 insensitivity, a characteristic observed in many human diseases including endometriosis, endometrial cancer, and in women who suffer from miscarriages.

2.3 Regulation of PR Activity

Upon binding of P4, progesterone receptors undergo a conformational change causing them to dissociate from heat shock proteins and chaperones and dimerize, thus gaining affinity for various coregulators and DNA response elements found within the genes they transcribe. One of the most well studied mediators of PR transcription is the p160/SRC (steroid receptor coactivator) family of coregulators, of which the three members are *SRC-1*, *SRC-2* and *SRC-3*. These coregulators interact with and bind to the LBD of ligand-bound steroid hormone receptors and have been shown to modulate the activity of nuclear receptors including PR *in vitro* [Hofman et al., 2002]. *SRC-1* and *-2* are expressed in both the

epithelial and stromal endometrium while SRC-3 expression is not detected in the preimplantation mouse uterus [Jeong et al., 2007].

Given the expression pattern of these molecules, one might predict that SRC-1 and -2 are the prime cofactors of PR transcription and indeed such is the case, with SRC-2 being the preeminent PR coactivator. SRC-1 knockout females are fertile but do exhibit a dampened response to exogenous steroid hormone treatment including a reduced increase in uterine size upon E2 treatment [Xu et al., 1998]. Additionally, SRC-1^{-/-} females reveal a reduction in stromal differentiation when treated to undergo an artificially induced decidual response. Accordingly, although SRC-1 null females were fertile, SRC-1 plays an important role in mediating the response of the mouse uterus to steroid hormones.

Global ablation of SRC-2 resulted in a partial lethality phenotype accompanied by metabolic defects [Chopra et al., 2008; Gehin et al., 2002]. In SRC-2 null females that did survive to adulthood, there was an observed partial reduction in the artificial decidualization reaction as well as a hypofertility phenotype due to placental defects [Gehin et al., 2002; Jeong et al., 2007]. To circumvent the partial lethality and metabolic phenotypes that preclude effective adult uterine studies, a conditional ablation of SRC-2 was achieved by crossing a mouse model with floxed (flanked by loxP) SRC-2 alleles with the PR-Cre mouse model. [Mukherjee et al., 2006; Soyak et al., 2005]. This model excises alleles in tissues that express PR (pituitary gland, mammary gland, oviduct, corpus luteum of the ovary and all compartments of the murine uterus) as demonstrated when crossed with the Rosa26R reporter line which expresses the *lacZ* reporter gene in all tissues with Cre recombinase activity [Soriano 1999]. The mice generated from this cross (SRC-2^{d/d}) were infertile and exhibited a reduction in the artificial decidualization reaction [Mukherjee et al., 2006]. Furthermore, microarray analysis has shown SRC-2 to be involved in *in vivo* P4-mediated gene transcription [Jeong et al., 2007]. These data demonstrate that SRC-2 is an integral mediator of pregnancy and P4 signaling.

Considering the similar phenotypes and overlapping expression profiles of SRC-1 and SRC-2, it was speculated that some form of functional compensation may occur between these coregulators. Hence, the SRC-2 conditional knockout mouse was crossed into the SRC-1^{-/-} mouse model [Mukherjee et al., 2006]. Mice lacking both uterine SRC-1 and -2 were sterile with a complete ablation of decidualization, demonstrating that both of SRC-1 and -2 are necessary for decidualization. These mouse models are in contrast with the SRC-3 knockout mouse which is capable of a normal decidual response, although females are slightly subfertile [Jeong et al., 2007].

Distinct from the p160 family of coregulators, PR can also recruit secondary transcription factors such as *Klf9* to act as cofactors. Krüppel-like factor (KLF)-9 is a transcription factor that has been shown to directly interact with and coactivate PR both *in vitro* and *in vivo* [Zhang et al., 2002; Zhang et al., 2003]. KLF9 expression is detected in the preimplantation stroma and its ablation results in uterine hypoplasia and female subfertility due to a reduction in the number of implantation sites [Simmen et al., 2004]. Although *Klf9* is expressed in the stroma, it has been shown that it can effect the response of adjacent luminal epithelial cells to P4 and that null females exhibit a partial P4 resistance [Simmen et al., 2004; Velarde et al., 2005]. Recently, it was demonstrated that KLF9 and bone morphogenic protein BMP2, a well known regulator of implantation, undergo cross-regulation in order to establish and maintain uterine receptivity [Lee et al., 2007; Pabona et al., 2010].

3. Effectors of PR Action

P4 and PR regulate implantation and decidualization via the activation of a cascade of various molecules. Periimplantation PR is known to propagate its signal by inducing the

expression of many ligands and thus multiple signaling pathways. Some examples of these ligands that have been shown to play an important role in fertility include *Ihh*, *Bmp2*, wingless-related MMTV integration site 4 (*Wnt4*), and *ErbB* ligand heparin-binding epidermal growth factor (*Hb-EGF*) [Lee et al., 2006; Lee et al., 2007; Li et al., 2007; Takamoto et al., 2002; Wang et al., 1994; Xie et al., 2007]. Either by virtue of ligand induction or by other means, PR can also affect signaling by inducing the expression of signal modulators and/or transcription factors. Examples of the aforementioned molecules include adaptor proteins like mitogen inducible gene 6 (*Mig6*), cell cycle regulators like cyclin D3 and *p53*, and transcription factors like CCAAT/enhancer binding protein beta (*C/EBPβ*), *Hand2*, and chicken ovalbumin upstream promoter-transcription factor II (*COUP-TFII*). In the following section we highlight several of the known effectors and propagators of PR signaling.

3.1.1 Signaling Ligands Induced by PR and the Pathways they Regulate—

Multiple approaches aimed at identifying P4 regulated genes using high density DNA microarrays have been conducted, including the employment of PRKO mice or PR antagonist RU486 [Cheon et al., 2002; Jeong et al., 2005] (as reviewed in [Lee et al., 2006]). *Ihh*, a member of the hedgehog family of morphogens, is one such P4-induced gene identified in this fashion [Matsumoto et al., 2002; Takamoto et al., 2002]. Interestingly, while *Ihh* expression is restricted to the endometrial epithelium and is observed with increasing intensity just prior to the time of implantation, its downstream effectors Patched-1 (*Ptch1*) and COUP-TFII are expressed in the endometrial stroma during this same period [Takamoto et al., 2002]. The expression pattern of these molecules alludes to a potential role of *Ihh* in the mediation of paracrine P4 signaling between the uterine epithelial and stromal compartments. Embryonic and perinatal lethality phenotypes observed in *Ihh* null mice prohibit the study of *Ihh* in the adult uterus [St-Jacques et al., 1999]. To bypass the observed lethality, conditional ablation of *Ihh* was achieved by crossing a floxed allele with the PR-Cre mouse model [Razzaque et al., 2005]. The resulting *Ihh^{d/d}* females phenocopied the uterine defects observed in PRKO females, with an inability to support attachment, implantation, and decidualization [Lee et al., 2006]. Additionally, similar to the defects and elevated *Ltf* expression observed in *Fkbp4^{-/-}* mice, the attachment defect observed in *Ihh^{d/d}* females was shown to be due to a failure to attenuate E2 signaling in the preimplantation luminal epithelium as indicated by elevated expression of the secreted glycoprotein MUC-1 [Surveyor et al., 1995]. These results convey that *Ihh* is a major mediator of PR action in the uterus. Through findings of multiple studies, it's been shown that *Ihh* mediates decidualization and implantation via the activation of multiple signaling pathways including the *Ihh-Ptch1-COUP-TFII-Bmp2-Wnt4* signaling axis and *ErbB* signaling [Kurihara et al., 2007; Lee et al., 2006; Lee et al., 2007].

Although it's not a ligand, COUP-TFII is one of the downstream mediators of *Ihh* signaling and its expression is lost in *Ihh^{d/d}* females [Lee et al., 2006]. Although mice containing null alleles for *COUP-TFII* are embryonic lethal, a portion of female *COUP-TFII^{+/-}* heterozygotes are viable and exhibit reduced fecundity attributed to both ovarian and uterine defects [Takamoto et al., 2005]. Given that PR is expressed in the ovarian granulosa cells while COUP-TFII is expressed in the theca cells, this differential expression permitted the utilization of the PR-Cre mouse model to further address the uterine specific defects observed in *COUP-TFII^{+/-}* mice [Soyal et al., 2005; Takamoto et al., 2005]. The resulting *COUP-TFII^{d/d}* mice resemble the conditional ablation of *Ihh* with failures in attachment and decidualization [Kurihara et al., 2007]. Also similar to *Ihh^{d/d}* mice, conditional ablation of *COUP-TFII* results in enhanced E2 activity in the uterine epithelium as indicated by elevated levels of ERα, phosphorylated ERα (the active form) and ER target genes *Ltf*, *Muc1*, *C3*, and *Clca3* [Braga and Gendler 1993; DeMayo et al., 2002; Driggers and Segars

2002; Jeong et al., 2006; Lagow et al., 1999; Sundstrom et al., 1989; Surveyor et al., 1995]. The suppression of ER α activity in the uterine epithelium was later shown to be one of the critical functions of COUP-TFII. The administration of the pure ER α antagonist ICI 162,780 was able to restore the defects observed in the conditionally ablated *COUP-TFII* females in attachment and decidualization, albeit to a lesser extent than what is normally observed in control mice [Lee et al., 2010]. These data indicate that one of the primary functions of COUP-TFII is the regulation of the window of receptivity by suppression of epithelial ER α activity.

3.1.2 Bmp2 Signaling—In addition to the role of COUP-TFII in E2 regulation, one of its functions is the induction of known decidual regulator *Bmp2*. *Bmp2* is a member of the BMP family of morphogens. Its expression is observed in the uterine stroma near the site of attachment and persists through the early phases of decidualization [Paria et al., 2001; Ying and Zhao 2000]. BMP2 expression was shown to be lost in the stroma of *COUP-TFII*^{d/d} uteri. Furthermore, *Bmp2* was demonstrated to be downstream from the *Ihh-COUP-TFII* signaling axis functionally and genetically. When recombinant BMP2 was administered at the time of the application of the decidual stimulus, a marked rescue of the induction of uterine weight gain and differentiation, as indicated by alkaline phosphatase staining, could be observed in the stimulated uterine horns of *COUP-TFII* conditional mutants [Kurihara et al., 2007]. *Bmp2* is yet another gene in which global ablation results in embryonic lethality and thus investigation of its functions in the adult uterus were executed using the PR-Cre mouse model [Lee et al., 2007; Zhang and Bradley 1996]. Female *Bmp2*^{d/d} mice were infertile, however, unlike conditional *Ihh* or *COUP-TFII* mutants, blastocysts were able to attach to the luminal epithelium. The block in pregnancy progression was shown to occur due to a failure in implantation and subsequent decidualization when induced by either blastocysts or artificial stimulus [Lee et al., 2007]. Moreover, the expression of the *Fkbp* family of chaperones, including *Fkbp-3*, *-4*, and *-5*, was significantly reduced in *Bmp2*^{d/d} females, indicating that *Bmp2* may play an important role in maintaining P4 action during the postimplantation period by mediating a feed-forward loop of progesterone signaling. In addition to the critical role of *Bmp2* in murine decidualization, it has been shown to play an important role in the *in vitro* decidualization of human endometrial stromal cells. The use of siRNA to attenuate *Bmp2* expression prevents decidualization while the addition of recombinant BMP2 to the hormonal decidualization cocktail enhances it [Li et al., 2007].

3.1.3 Wnt Signaling—In investigating the mechanism by which *Bmp2* regulates decidualization, microarray analysis revealed that the induction of *Wnt* ligands *Wnt4* and *Wnt6* was severely attenuated in the decidual-stimulated uterine horns of *Bmp2*^{d/d} mice. WNT proteins are a highly conserved family of secreted glycoproteins that are critical for multiple processes including cell-cell communication, cell growth, and differentiation (reviewed in [Wodarz and Nusse 1998]). WNT signaling has been implicated in both uterine postnatal differentiation and the regulation of embryo implantation [Jeong et al., 2009; Li et al., 2005; Mohamed et al., 2005; Xie et al., 2008]. Ablation of *Wnt7a* or *Wnt5a* as well as conditional ablation of β -catenin results in altered postnatal uterine development while mice with an ablation of *Wnt4* fail to develop the Mullerian duct, the precursor structure to the uterus [Jeong et al., 2009; Mericskay et al., 2004; Miller and Sassoon 1998]. Additionally, *Wnt4*^{-/-} mice exhibit perinatal lethality due to kidney failure [Stark et al., 1994]. *Wnt4* expression in the adult uterus is under the regulation of E2 and occurs at specific times throughout pregnancy. Although its expression can be weakly detected in the luminal epithelium at day 0.5 of pregnancy, its primary expression appears to be in the uterine stroma at day 4.5, persisting into the decidua [Daikoku et al., 2004; Hayashi et al., 2009; Hou et al., 2004; Katayama et al., 2006]. In addition to a potential role of *Wnt4* in the regulation of murine pregnancy, it has been implicated in human decidualization as the use

of siRNA to attenuate its expression was shown to prevent *in vitro* decidualization [Li et al., 2007].

Recently, *Wnt4* was conditionally ablated using the PR-Cre mouse model and these *Wnt4^{d/d}* females were severely subfertile and exhibited defects in postnatal uterine development [Boyer et al., 2010; Franco et al., 2010]. Upon conditional ablation of *Wnt4*, a reduction in the number of uterine glands as well as the focal accumulation of a layer of cells around the luminal epithelium expressing the basal cell marker p63 was observed [Koster and Roop 2007]. Furthermore, the observed focal stratification was exacerbated by prolonged exposure to E2, eventually developing to squamous cell metaplasia in a processes similar to what has been observed in humans who have been exposed *in utero* to the synthetic estrogen diethylstilbestrol (DES) [Goldberg and Falcone 1999]. The defects in fertility could possibly have been attributed to the lack of uterine glands in this model since their secretion of leukemia inhibitory factor (LIF) is critical for implantation and decidualization [Jeong et al., 2010; Stewart et al., 1992]. However, unlike other models in which LIF deficiency is a driver of infertility, the administration of recombinant LIF was unable to rescue the artificial decidualization response, indicating that uterine defects are the primary source of infertility in these mice [Chen et al., 2000; Jeong et al., 2010].

Although the uteri of *Wnt4^{d/d}* female mice are able to support normal attachment, many blastocysts failed to invade through the luminal epithelium and induce a decidual response. Like *Bmp2^{d/d}* conditional mutant females, *Wnt4^{d/d}* females show a failure to induce uterine weight gain and differentiation following artificial decidualization; however, there is not a defect in decidual proliferation in these mice. In this case, the observed decidualization defects were attributed to mislocalization of FOXO1, resulting in elevated levels of apoptosis. When FOXO1 is found in the nucleus, it promotes the transcription of many proapoptotic genes [Huang and Tindall 2007]. Under the influence of P4, FOXO1 is normally shuttled to the cytoplasm, silencing expression of its target genes [Brosens and Gellersen 2006; Labied et al., 2006]. *Wnt4* regulation of decidualization as well as FOXO1 localization is due in part to reduced expression of *Fkbp4*. Thus, *Wnt4* is an important regulator of decidualization by mediating P4 actions to promote both stromal cell differentiation and survival.

3.1.4 P4 Regulation of Erbb Ligands and Subsequent Decidualization—It is well established that the uterus is a steroid hormone responsive organ and that crosstalk between growth factor and steroid hormone signaling occurs [Ignar-Trowbridge et al., 1992]. The Erbb family of growth factors and receptors is one such signaling family that has been increasingly implicated in female fertility [Lim et al., 1998]. *Hb-EGF* and *Areg* are two of the ligands that act on this pathway and both are regulated by P4 while *Hb-EGF* is also regulated by E2 [Das et al., 1995; Wang et al., 1994]. Uterine *Hb-EGF* expression is induced by blastocysts and can be found in the luminal epithelium just prior to attachment. Its expression can subsequently be observed in the proliferating stromal cells during the postimplantation period and persists through to the secondary decidual zone [Das et al., 1994]. When *Hb-EGF* was ablated either globally or conditionally with the PR-Cre, female mice were found to have reduced litter sizes with an inability to support implantation [Xie et al., 2007]. Mechanistically, *Hb-EGF* has been shown to regulate decidualization by upregulating the Cyclin D3-mediated polyploidization of uterine stromal cells [Das et al., 1999; Tan et al., 2002; Tan et al., 2004]. Interestingly, *Areg* expression was elevated in mice lacking *Hb-EGF*, suggesting that the partial uterine phenotype observed in these mice may be due to compensation by other Erbb ligands [Xie et al., 2007].

Areg expression is found transiently in the luminal epithelial cells at the time of implantation [Das et al., 1995]. However, *Areg^{-/-}* female mice did not exhibit defects in

fertility, further supporting the notion that there may be redundancy in the ligands [Luetke et al., 1999]. Furthermore, mice null for *ErbB* ligands *EGF* or *epiregulin* also did not display fertility defects, indicating that while ablation of *Hb-EGF* renders female mice subfertile, ablation of no single gene is sufficient to cause complete infertility [Lee et al., 2004; Luetke et al., 1999]. The potential for redundancy amongst the *ErbB* ligands suggest that perhaps the receptors may be the rate-limiting factor.

ErbB signaling requires ligand-dependent dimerization of the receptors (*ErbB1-4*) as either homo- or heterodimers, with different ligands exhibiting preferential binding of particular receptors [Ullrich and Schlessinger 1990]. It's known that HB-EGF exerts its effects by binding to ERBB1 (EGFR) and ERBB4. *Egfr* expression is observed primarily in the subepithelial stroma while *ErbB4* expression is found primarily in the submyometrial stroma and myometrial connective tissues, suggesting that *Egfr* might be the predominant receptor of *Hb-EGF* mediated implantation and decidualization [Lim et al., 1998]. Further support for a potential role of *Egfr* in female fertility exists in that P4 has been shown to maintain EGFR expression during pregnancy and promote growth of the decidua [Dai and Ogle 1999; Ogle et al., 1999]. Notably, EGFR expression in the subepithelial stroma was reported to be reduced in *Ihh^{d/d}* females [Lee et al., 2006]. While compelling data suggests *Egfr* likely plays an important role in female reproduction, bona fide *in vivo* evidence of a critical function is currently lacking due to the observed embryonic or perinatal lethality observed in *Egfr^{-/-}* mice, depending on genetic background [Threadgill et al., 1995].

3.2 Progesterone Regulation of Signaling Modulators

Separate from the ability of P4 to induce ligands and their signaling pathways, multiple reports demonstrate that P4 can induce the expression of genes such as *Mig6*, Cyclooxygenase 2 (*Cox2*) and many cell cycle regulatory proteins which can all influence active signaling paradigms. ERBB receptor feedback inhibitor 1 (*Erff1*, *Mig-6*) is an adaptor protein containing multiple protein interaction domains including a Cdc42/Rac interactive binding (CRIB) domain, Src homology 3 (SH3) binding domain, and a 14-3-3 binding domain [Burbelo et al., 1995; Makkinje et al., 2000; Pirone et al., 2001]. It can be induced by various mitogens and can act as a negative feedback inhibitor of EGFR signaling [Anastasi et al., 2003; Fiorentino et al., 2000; Fiorini et al., 2002; Hackel et al., 2001]. *Mig6* was identified as a P4 responsive gene using microarray analysis [Jeong et al., 2005]. Its expression was later shown to also be induced by E2 alone and synergistically by cotreatment of E2 and P4. In the preimplantation mouse uterus, *Mig6* expression is detected in both the epithelium and stroma beginning at 2.5 days post coitum (dpc) [Kim et al., 2010]. Interestingly, *Mig6* is involved in regulatory loop that feeds back to regulate P4 signaling. *Mig6* null mice exhibit an inability of P4 to inhibit E2-induced uterine weight gain and target gene expression [Jeong et al., 2009]. Furthermore, ovariectomized *Mig6^{-/-}* mice develop endometrial hyperplasia, which when challenged with exogenous E2, developed into invasive endometrioid-type endometrial adenocarcinoma. When *Mig6* was conditionally ablated with the PR-Cre, E2 hypersensitivity similar to that which was observed in *COUP-TFII^{d/d}* mice resulted in a block in implantation, rendering these mice infertile. The results from these studies demonstrate that *Mig6* modulates the ability of P4 signaling to regulate ER activity.

3.3 Progesterone Mediation of Implantation via Prostaglandin Regulation

Following blastocyst attachment and throughout implantation there is an increase in endometrial stromal vascular permeability and this process is known to be mediated by prostaglandins (PG) [Chakraborty et al., 1996]. Prostaglandin-endoperoxide synthase 1 and -2 (*Ptgs-1*, *Cox1*; *Ptgs-2*, *Cox2*) are two enzymes that mediate the synthesis of PGs, and PG receptors have been shown to be induced by P4 [Lim et al., 1999]. While *Cox1* is a

constitutive enzyme and thought to play a basic housekeeping role, *Cox2* is inducible and is found in the luminal epithelium and in underlying stromal cells at the site of blastocyst attachment [Lim et al., 1997]. Consistent with the expression pattern of these two enzymes, *Cox1*^{-/-} females are fertile. *Cox2*^{-/-} females are infertile with defects in ovulation, implantation and decidualization [Lim et al., 1997]. Although, in a background dependent fashion, *Cox1* is able to compensate for *Cox2* expression, improving fertility [Wang et al., 2004]. Furthermore, the use of selective inhibitors of either COX1 or COX2 separately had no significant effect on blastocyst implantation, but a combination of both inhibitors in lower doses resulted in a delay and impairment in implantation, ultimately leading to significant pregnancy loss. Administration of higher doses of inhibitors further reduced PG production and completely prevented implantation [Pakrasi and Jain 2007]. Lastly, it's been shown that uterine angiogenesis during decidualization is severely compromised in *Cox2*^{-/-} mice, and that this is due to a loss of coordination and regulation of vascular endothelial growth factor (*VEGF*) signaling in the absence of COX2 derived PGs [Douglas et al., 2009; Matsumoto et al., 2002]. These results demonstrate the critical role of both angiogenesis and prostaglandin production during implantation.

3.4 Transcription Factors Regulated by P4

The final class of molecules highlighted in this review enlisted by P4 to execute the pregnancy paradigm is transcription factors. The ultimate biological aim of the uterus is the support and nourishment of the embryo, a process that requires the production of many different proteins. A few prominent examples of transcription factors that mediate this process that have not been elaborated upon here previously include *Hoxa10*, *C/EBPβ* and *Hand2*.

3.4.1 Hoxa10—Homeobox (Hox) genes are a multigene family of developmentally regulated transcription factors that are well known to display distinct spatial and temporal patterns of expression [Krumlauf 1994]. Homeobox A10 (*Hoxa10*), a member of the AbdB-like Hoxa gene family, is expressed in the uterine luminal and glandular epithelium from days 0.5 to 2.5, after which it shifts to the subepithelial stroma, persisting robustly in the stroma through decidualization to day 7.5 [Satokata et al., 1995]. This expression pattern along with the observation that *Hoxa10* is induced by P4 implicated it might be involved in adult uterine function [Ma et al., 1998].

Indeed, *Hoxa10*^{-/-} females were found to be subfertile with defects in attachment and decidualization. In investigating the cause of decreased fertility it was determined that most of the embryos found at the time of implantation were either degenerating or delayed in development. Approximately half of these embryos were unattached and those that did attach resulted in abnormal implantation sites due to defects in decidualization and stromal-cell proliferation [Benson et al., 1996; Lim et al., 1999; Satokata et al., 1995]. Furthermore, it was determined that a subset of P4 regulated genes, including *Cox2* and prostaglandin receptors, were deregulated, indicating that *Hoxa10* is required for successful implantation as a partial mediator of P4 signaling [Lim et al., 1999].

3.4.2 CCAAT/Enhancer Binding Protein β (C/EBPβ)—CCAAT/enhancer binding protein β is a member of the basic leucine zipper (bZIP) family of transcription factors that regulate diverse processes such as cell proliferation, differentiation and apoptosis [McKnight 2001; Ramji and Foka 2002; Wedel and Ziegler-Heitbrock 1995]. C/EBPβ is regulated by both ER and PR and mediates the actions of E2 and P4 during the periimplantation period. The expression of C/EBPβ is detected in the stroma on day 4.5 and remains robustly expressed in the decidua until day 9.0 [Bagchi et al., 2006; Mantena et al., 2006]. *C/EBPβ*^{-/-} females are infertile with defects originating in both ovarian and uterine functions [Sterneck

et al., 1997]. Uterine deficiencies were attributed to a failure in decidualization including impairments in proliferation and differentiation of the uterine stromal cells. Furthermore, *C/EBP β* null mice displayed a reduction in E2-induced epithelial cell proliferation [Bagchi et al., 2006]. Recent work elucidating the mechanism by which *C/EBP β* induces stromal cell proliferation indicates that the transcription factor plays a key role in the regulation of the G2 to M cell cycle checkpoint. It was shown that *C/EBP β* binds directly to the promoter of cyclin B2 approximately 1kb upstream of the transcriptional start site and induces its expression in the presence of decidualogenic stimuli. Although *C/EBP β* ^{-/-} uterine stromal cells are able to progress through the S phase and replicate their DNA, reduced expression of proliferative molecules cyclin B2 and *cdc25C* accompanied by elevated expression of p53 and cell cycle inhibitors p21 and p27 resulted in a block at the G2 to M transition [Wang et al., 2010]. Altogether, these results demonstrate that *C/EBP β* is a key mediator of stromal cell proliferation and thus decidualization by regulating the expression of multiple cell cycle regulatory proteins.

3.4.3 P4 Inhibition of Epithelial Proliferation Mediated by Hand2—Several of the mouse models discussed here including those involving *Ihh*, *COUP-TFII* and *Mig6* demonstrate that the attenuation of E2 activity in the uterine luminal epithelium by P4 is an absolute requirement for successful implantation. Although the physiological necessity of this event is well understood, the molecular mechanism by which it occurs has been unclear. Key findings of recent work illuminate a molecular pathway in which *Hand2* conveys the antiproliferative effects of P4. *Hand2* is a basic helix-loop-helix transcription factor known to play a critical role during cardiac morphogenesis (reviewed in [Srivastava 1999]). It was identified as a potential PR regulated gene by a microarray experiment in which RU-486 was administered to periimplantation mice to identify P4 pathways mediating implantation [Bagchi et al., 2005]. This notion was directly confirmed by the observation that treatment of mice with exogenous P4 stimulated robust induction of *Hand2* in the uterine stroma. Conditional ablation of *Hand2* using the PR-Cre resulted in infertility due to a failure in blastocyst attachment resulting from elevated epithelial E2 activity [Li et al., 2011]. Following gene expression profiling studies using stromal cells isolated from day 4 pregnant mice, it was determined that *Hand2* functions to suppress stromal expression of multiple members of the fibroblast growth factor (FGF) family. It was then shown that stromal-derived FGFs act in a paracrine fashion, stimulating their receptors in the luminal epithelium and thus resulting in an ERK 1/2 mediated activation of ER α via phosphorylation [Li et al., 2011].

This work provides clear molecular details regarding the mediation of the antiproliferative effects of P4. However, it would be interesting to determine how other mouse models with similar phenotypes fit into this pathway. Given that the induction of *COUP-TFII* in the uterine stroma by P4 mediated expression of epithelial *Ihh* is also critical for the inhibition of epithelial E2 activity, one might speculate that these molecules are intertwined in some fashion with *Hand2*. Also, microarray data examining *Ihh*^{d/d} uteri show reduced expression of *Hand2* [Franco et al., 2010]. Furthermore, *Mig6* is not only induced by P4 and expressed in both the epithelium and stromal compartments, but it has been shown to inhibit signaling pathways that activate ERK 1/2 [Fiorentino et al., 2000; Fiorini et al., 2002]. The potential for interactions and regulatory networks between these proteins warrants further investigation.

4. Conclusion and Future Challenges

Progesterone has long been considered the hormone of pregnancy [Allen 1970]. Advancements in technology and the use of mouse models have extensively enhanced our understanding of the molecular events regulating early pregnancy. The progesterone

receptor exerts pleiotropic effects by regulating multiple types of proteins including ligands, receptors, chaperones, signaling proteins and transcription factors (Figure 1). Understanding these processes is not only vital to advancements in reproductive physiology but also in pathology, for these same signaling pathways go awry in diseases like endometriosis and endometrial cancer.

However, with an accelerating influx of data, an equal number of questions to be addressed arise. The eminent importance of the paracrine signaling and epithelial-stromal cross talk in the regulation of early pregnancy is made obvious by observations in many of the mouse models listed within this review. Although the use of the PR-Cre mouse model has been tremendously successful in the creation of conditional knockouts and advancements in our understanding, there is an imploring necessity for the generation of uterine compartment-specific mouse models expressing Cre-recombinase. Several models expressing Cre under the regulation of promoters specific to particular uterine compartments have recently been developed (Wnt7a and Sprr2f- epithelium; Amhr2- mesenchyme; Myh11- myometrium); however, depending on the functions of the gene of interest, the presence of Cre-recombinase activity in extrauterine tissues in these models has the potential to preclude effective uterine studies [Contreras et al., 2010; Jamin et al., 2002; Regan et al., 2000; Winuthayanon et al., 2010]. The advent of Cre models expressed solely in the uterus exhibiting compartment specificity would allow researchers to investigate important questions concerning proteins expressed in multiple compartments.

Additionally, it's well established that PR regulates a plethora of proteins, but it's likely there are many novel PR targets that regulate important functions that have yet to be identified. The use of chromatin immunoprecipitation coupled with DNA sequencing (ChIP-seq) will help identify PR target genes and the different hormonal conditions that drive PR recruitment to the promoters of each of these genes. The use of proteomics is needed to advance our understanding of what proteins PR is interacting with and thus driving its specificity and activity. A systems approach combining gene expression profiling by microarrays, transcription factor binding sites by ChIP-seq, and protein interactions using proteomics would be immensely valuable in the advancement of our understanding of PR target gene regulation. Lastly, the use of biomarkers to identify disease is prevalent in many other tissues. The identification of biomarkers indicative of infertility and malignant disease would be exceedingly beneficial in the diagnosis and treatment of these incapacitating conditions. The use of primary stromal cell culture has been the predominant method of interrogating gene function in human biology. While several techniques have begun to be utilized, including 3D culture of human endometrium and renal capsule xenograft experiments, the use of these systems has been sparing thus far. Hence, there remains a pressing demand for the development of tools that facilitate translation of findings from mice to humans.

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Highlights

- In this review we discuss mediators and effectors of progesterone signaling
- We collate data and insights gained from many mouse models
- We discuss progesterone regulation of proliferation and differentiation
- We examine how progesterone regulates implantation and uterine decidualization

Figure 1. Schematic highlighting the complex network of genes enlisted by PR during periimplantation uterine biology. $\rightarrow$ Indicates activation or induction. $\dashv$ indicates inhibition or repression. Dashed arrows represent potential induction lacking bona fide evidence.

Table 1
Uterine Phenotypes of Genes that Mediate Progesterone Signaling During Early Pregnancy

Molecule	Protein	Expression	Fertility	Att	Impn	Dec	Ref
Esr1	TF, SR	LE, GE, S, M	Infertile	No	No	Imp	[26]
Esr2	TF, SR	LE, GE, S, M	Fertile	n/a	n/a	n/a	[67]
PR-A	TF, SR	LE, GE, S, M	Infertile	No	No	No	[21]
PR-B	TF, SR	LE, GE, S, M	Fertile	n/a	n/a	n/a	[21]
Fkbp4	Chaperone	S	Infertile	No	No	No	[131, 148]
Fkbp5	Chaperone	S	Fertile	n/a	n/a	n/a	[131]
SRC-1	Co-reg	LE, GE→S	Fertile	n/a	n/a	Imp	[147]
SRC-1/2 ^{+/+}	Co-reg	LE, GE→S	Infertile	n/a	n/a	No	[98]
SRC-2	Co-reg	LE, GE→S	Sub/Infertile*	n/a	No	Imp	[58, 98]
SRC-3	Co-reg	M	Subfertile	n/a	n/a	Yes	[58]
C/EBP β	TF	S	Infertile	Yes	Yes	No	[5, 120]
COUP-TFII	TF	S	Sub/Infertile*	No	No	No	[69, 125]
Foxo1a	TF	S	n/a	n/a	n/a	n/a	n/a
Hand2	TF	S	Infertile	No	No	No	[81]
Hoxa10	TF	LE/GE→S	Subfertile	Imp	No	No	[85]
Klf9	TF	S	Subfertile	n/a	Imp	n/a	[113]
Areg	Ligand	LE	Fertile	n/a	n/a	n/a	[31, 86]
Bmp2	Ligand	S	Infertile	Yes	No	No	[78]
HB-EGF	Ligand	LE/GE→S	Subfertile	No	No	n/a	[30, 145]
Ihh	Ligand	LE, GE	Infertile	No	No	No	[75]
Wnt4	Ligand	LE→S	Subfertile	Yes	No	Imp	[41]
Mig6	Adaptor	LE, GE, S	Infertile	No	No	Yes	[64]
Cox1	Enzyme	LE	Fertile	n/a	n/a	n/a	[136]
Cox2	Enzyme	LE→S	Infertile	No	No	No	[83, 136]

Abbreviations: Att: Attachment; Inn: Implantation; Dec: Decidualization; Ref: Reference TF: Transcription Factor; SR: Steroid Receptor; Co-reg: Coregulator; LE: Luminal Epithelium; GE: Glandular Epithelium; S: Stroma; M: Myometrium; Imp: Impaired; N/A: data not found

* Straight knockout or heterozygote subfertile, conditional knockout infertile

→ Indicates transitioning expression between compartments

⁷ Double knockout

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